Date: 9/1/14 People in lab: Kira Buckowing, Joshua Heath

Title: nosZ PCR and making competent cells

Start Time: 12:30 pm

Purpose: To have chemically competent cells so the project can progress and to test the odd results from the previous PCR's of nosZ

Protocol: LTM Ed.

Exceptions: 2uL DNA and 2.5 uL primers used

Products:

Sample Label	Description	Source Label	Quantity
PCR1 9/1	Genomic prep	GP1 8/16	1
PCR2 9/1	Resuspended PAO1 Cells	PAO1 Temp 8/16	1

Results:

Notes:

Stop Time: 8:15 pm

Next: Gel extraction of PCR products and testing of comp cells

Date: 9/2/14 People in lab: Kira Buckowing

Title: Chemical Transformations

Start Time: 3 pm

Purpose: Test newly made competent cells and to get hmp colonies

Protocol: LTM Ed. 2 Chemical Transformation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
CT1 9/2	20 uL	L1 8/13	1
CT2 9/2	200 uL	L1 8/13	1
CT3 9/2	20 uL	RFP in chlor bb 12/18/13	1
CT4 9/2	200 uL	RFP in chlor bb 12/18/13	1

Results: Growth on CT 3 and CT4 so comp cells work

Notes:

Stop Time: 6:30 pm

Next:

Date: 9/16/14 People in lab: Kelsey Crossen

Title: Ligation of hmp and pro+RBS backbone

Start Time: 2:05 pm

Purpose: To ligate hmp into vector for transformation into comp cells

Protocol: LTM Ed. 2 Ligation

Exceptions: L1 - 2 uL vector, 6 uL insert; L2 = 4 uL vector and insert; L3 = 2 uL insert and 4 uL vector

Products:

Sample Label	Description	Source Label	Quantity
9/16 L1	hmp and pro+RBS	8/11 GE1 and 8/13 GE1	1
9/16 L2	hmp and pro+RBS	8/11 GE1 and 8/13 GE1	1
9/16 L3	hmp and pro+RBS	8/11 GE1 and 8/13 GE1	1

Results:

Notes:

Stop Time: 2:35 pm

Next: Chemical transformations

Date: 9/19/14 People in lab: Kelsey Crossen, Kira Buckowing

Title: Transforming hmp Ligations

Start Time: 2 pm

Purpose: To transform ligated hmp/vector into comp cells for liquid cultures and minipreps

Protocol: LTM Ed. 2 Chemical Transformations

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
CT1 9/19	20 uL	L1 9/16	1
CT2 9/19	200 uL	L1 9/16	1
CT3 9/19	20 uL	L2 9/16	1
CT4 9/19	200 uL	L2 9/16	1
CT5 9/19	20 uL	L3 9/16	1
CT6 9/19	200 uL	L3 9/16	1

Results: (9/21) No growth

Notes: Stop Time: 5 pm

Next:

Date: 9/25/14 People in lab: Kelsey Crossen

Title: Digestions of hmp and vectors

Start Time: 2 pm

Purpose: Digest hmp from TOPO vector, chlor bb with pro+RBS and iGEM plasmid (psB1C3) **Protocol:** LTM Ed. 2 Digestion

Exceptions: psB1C3 digestion done at half value due to low concentration

Products:

Sample Label	Description	Source Label	Quantity
D1 9/25	hmp digested with E and P	MP4 7/30	1
D2 9/25	hmp digested with E and P	MP4 7/30	1
D3 9/25	pro+RBS bacbone digested with S and P	MP2 8/9	1
D4 9/25	shipping backbone digested with E and P	2013 psB1C3	1

Results:

Notes: D1 needs to be ligated into D4 and D2 needs to be ligated into D3

Stop Time: 4:25 pm

Next:

Date: 9/26/14 People in lab: Kelsey Crossen

Title: Ligation of hmp into psB1C3 and pro+RBS backbones

Start Time: 2:15 pm

Purpose: To have successful ligated products

Protocol: LTM Ed. 2 Ligation

Exceptions: L2 and L3 have 4 uL of vector and insert

Products:

Sample Label	Description	Source Label	Quantity
L1 9/26	hmp in psB1C3	D1 and D4 9/25	1
L2 9/26	hmp in psB1C3	D1 and D4 9/25	1
L3 9/26	hmp in pro+RBS	D2 and D3 9/25	1
L4 9/26	hmp in pro+RBS	D2 and D3 9/25	1

Results:

Notes:

Stop Time: 2:50 pm

Next: Chemical Transformations

Date: 9/27/14 People in lab: Kelsey Crossen, Kira Buckowing

Title: Transformation of hmp into competent cells

Start Time: 10:10 am

Purpose: Transform ligation products into comp cells

Protocol: LTM Ed. 2 Chemical Transformations

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
CT1 9/27	20 uL	L1 9/26	1
CT2 9/27	200 uL	L1 9/26	1
CT3 9/27	20 uL	L2 9/26	1
CT4 9/27	200 uL	L2 9/26	1
CT5 9/27	20 uL	L3 9/26	1
CT6 9/27	200 uL	L3 9/26	1
CT7 9/27	20 uL	L4 9/26	1
CT8 9/27	200 uL	L4 9/26	1
CT9 9/27	20 uL	MP2 8/9	1
CT10 9/27	200 uL	MP2 8/9	1

Results: (9/28) Growth on all plates!

Notes: Tubes were incubated for a bit over 2 hours (~10-15 min)

Stop Time: 2 pm

Next: Start liquid culutures of any colonies that grow

Date: 9/28/14 People in lab: Kira Buckowing

Title: Innoculation of CT's 1-8 9/27

Start Time: 8:45 pm

Purpose: To grow liquid cultures for minipreps

Protocol: LTM Ed. 2 Innoculation

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
BC1 9/28	Innoculation	CT1 9/27	1
BC2 9/28	Innoculation	CT2 9/27	1
BC3 9/28	Innoculation	CT3 9/27	1
BC4 9/28	Innoculation	CT4 9/27	1
BC5 9/28	Innoculation	CT5 9/27	1
BC6 9/28	Innoculation	CT6 9/27	1
BC7 9/28	Innoculation	CT7 9/27	1
BC8 9/28	Innoculation	CT8 9/27	1

Results: (9/29) Only two of the innoculations grew, so the plates used were bad and the rest did not carry chlor resistance as needed

Notes:

Stop Time: 9:15 pm

Next: Minipreps

Date: 9/29/14 People in lab: Kira Buckowing

Title: Minipreps

Start Time: 12:15 pm

Purpose: To purify the wanted plasimd from liquid cultures

Protocol: LTM Ed. 2 Miniprep

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
MP1 9/29	Purrified hmp plasmid	BC1 9/28	1
MP2 9/29	Purified hmp plasmid	BC6 9/28	1

Results:

Notes:

Stop Time: 2:20 pm

Next: Digest of plasmid and gel to see if a vector and insert part are clearly visible

Date: 9/29/14 People in lab: Kelsey Crossen

Title: Nanodrop of miniprep product

Start Time: 3:15 pm

Purpose: Find out concentration of miniprepped hmp plasmids

Protocol: LTM Ed. 2 Nanodrop

Exceptions:

Products:

Sample Label	Description	Source Label	Quantity
---	ng/uL = 42.4; 260/280 = 2.13; 260/230 = 2.11	MP1 9/29	1
---	ng/uL = 26.2; 260/280 = 2.02; 260/230 = 1.61	MP2 9/29	1

Results:

Notes:

Stop Time: 3:30 pm

Next: Digest to confirm presence of hmp

Date: 9/29/14 People in lab: Kelsey Crossen

Title: Digest miniprep plasmids to confirm identity

Start Time: 3:30 pm

Purpose: To confirm the presence of hmp in the ligated plasmid

Protocol:

LTM Ed. 2 Digest and Gels

Exceptions:

Products:

Well	1	2	3	4	5	6	7	8
-	Ladder	-	D1	-	D2			

Products:

Results: Two bands visible for D1 ~1100 bp and ~2200 bp, correspondign to hmp and psB1C3 bacbone

Notes:

Stop Time: 5:50 pm

Next: Re-transform miniprep product into comp cells to make glycerol stocks